TABLE 1-continued

Buffers Used for Gellan Gum Gel Electrophoresis

Buffer	Composition	pH
TG	0.0039 mol/L TRIS and 0.047 mol/L glycine	8.3
BBE	0.022 mol/L bis (2-hydroxethyl) imino-tris (hydroxymethyl) methane, 0.045 mol/L boric acid, and 0.001 mol/L EDTA	6.8

Table 2 below shows the absorbance values for gellan gum gels in the ultraviolet range. The gels were dissolved in water (blank) formed using 5 mmol/L $CaCl_2$.

TABLE 2

Absorbance Values of Gellan Gum Electrophoresis Gels

Gel Concentration	240 nm	260 nm	280 nm	300 nm
0.05%	0.164	0.122	0.090	0.064
0.1%	0.234	0.176	0.130	0.088
0.2%	0.465	0.349	0.256	0.172

The low absorbance of the gels in Table 2 makes it possible to detect nucleic acids and proteins directly in the gel without staining. FIG. **1** shows the effect of incorporating additional polymers into the gellan gum electrophoresis gels. FIG. **1** is a graph illustrating the effect of polymer type and molecular weight on the electroosmotic flow in 0.2% gellan gum gels. The gels were cast with 5 mmol/L $CaCl_2$ and the buffer (0.0039 mol/L TRIS and 0.047 mol/L gylcine). Electrophoresis was at 2 V/cm for 4 h at 20° C. Electroosmotic flow was determined by measuring the mobility of cyanocobalamin. The polymers used were polyethylene oxide (PEO) or hydroxyethyl cellulose (HEC). The molecular weights were 5,000,000 (5M), 1,000,000 (1M), 250,000 (0.25M), 200,000 (0.2M), and 90,000 (90K).

As seen in FIG. **1**, increasing the concentration of additional polymer greatly reduces the electroosmotic flow in the gels. FIG. **1** shows that higher molecular weight polymers, such as polyethylene oxide (PEO), are more effective at reducing electroosmotic flow compared to lower molecular weight polymers. A variety of polymers both linear and branched can be incorporated into the gellan gum electrophoresis gels to modify their properties. Some examples of polymers which can be used include: dextran, Ficoll, amylose. alginates, amylopection, xanthan gum, Whelan gum, hydroxethyl cellulose, methyl cellulose, polyvinylpyrrolidone, and polyvinylalchol. FIG. **2** shows the effect of the increasing amount of PEO (5,000,000 molecular weight) on the separation of supercoiled (SC) and nicked circular (NC) plasmid DNA. The plasmids used were pBR 322 (4.3 kilobases), pDelta (8.0 kilobases), and pYA101 (13 kilobases). Electrophoresis was at 5 V/cm at 20° C. for 4 h. The buffer used was BBE (0.022 mol/L bis (2-hydroxethyl) imino-tris (hydroxymethyl) methane, 0.045 mol/L boric acid, and 0.001 mol/L EDTA). These results show that the mobility of the nicked circular form can be reduced to near zero while the supercoiled form has significant mobility. This allows for a very efficient separation between the two different physical forms of DNA.

EXAMPLE 2

DNA Electrophoresis Using Gellan Gum Formed Using Divalent Cations and a Diamine

Gel Formation and Electrophoresis

Gel formation was as described in Example 1. The gels were stained with ethidium bromide (1 μg/mL) with gentle mixing for 0.5 to 1 h in a solution of 0.1 mol/L KCI. The gels were destained for 0.5 h in a solution of 0.1 mol/L KCL.

Results

FIG. **3** shows the results of an electrophoresis gel made from gellan with the use of calcium cations. FIG. **3** is an image of a DNA electrophoresis gel using 0.1% gellan gum gel formed with 5 mol/L $CaCl_2$. The buffer was TB, (Table 1) with 1 mmol/L $CaCl_2$ and electrophoresis was at 140 V (4.7 V/cm) for 16 h at 20° C. In FIG. **3** the samples are: Lanes **1** and **3** a Kb Ladder; Lane **2**, a High Molecular Weight Ladder; Lanes **4** and **5**, a Hind III restriction digest of λ DNA; Lane **6** is a BstE II restriction digest of λ DNA; Lanes **7**, **8** and **9** are pBR 322 DNA (containing nicked circular and supercoiled forms); Lane **10** is a pBR 322 restriction digestion by Hind III; and Lane **11** is λ DNA (48.5 kilobase pairs). Numbers on the side of the picture indicate the size of DNA standards (lanes **1** and **2**) in kilobase pairs. This gel gave good resolution of DNA from about 50,000 base pairs to about 1,000 base pairs. The resolution of DNA below 1,000 base pairs requires the addition of polymers to the gels.

Gels formed with diamines gave similar results to those made with divalent cations. The formation of gels was dependent upon the protonation of both amines. A typical diamine such as ethylenediamine has values of 10.1 and 7.0 for the amino pK's. At a pH above 7, a significant fraction of the amine groups would not be protonated and gels would not form. DNA can be recovered from gellan gum gels made with diamines by raising the pH above 7. Gel slices returned to a solution when a buffer containing 10 mmo/L TRIS pH 8.0 was added.

A variety of diamines can be used to form gels. The basic amino acids, lysine, arginine, and histidine contain two positively charged amino groups, but did not form gels when added at concentrations of 5 mmol/L at pH below 7. The methyl esters (blocked carboxyl groups) of lysine, argiine, and histidine, all formed stable gels when added at concentrations of 5 mmol/L at pH below 7. The basic amino acids with unblocked carboxyl groups do not form gels apparently due to the proximity of the carboxyl group to the α amino group. EDTA can be added to gellan gum gels and buffers when gels have been formed with diamines. EDTA is commonly added to buffers used with DNA, because the removal of divalent metal cations by chelation prevents the activity of contaminating nucleases, if present. If contaminating nucleases are likely to be a problem, gels should be formed with diamines, and EDTA included in the buffer.

The addition of addition polymers to gellan gum electrophoresis gels had two effects on the separation of DNA. The polymers increased the resolution of lower molecular mass DNA and the electroosmosis was reduced. The decreased eletroosomotic flow allowed the electrophoresis to be done in a few hours. FIG. **4** shows the separation of DNA in a gellan gum electrophoresis gel containing 1.0% hydroxethyl cellulose (250,000 molecular weight) The gel in FIG. **4** was formed using DAHP (5 mmol/L) and contained HEC (1%). The running buffer was BBED containing 1 mmol/L DAHP, and 1.0% HEC. Electrophoresis was for 2.5 h at 300 V. Lane **1** is a λ DNA sample. Lane **2** is a Kb DNA ladder sample. Lane **3** is a 50 basepair Ladder DNA sample. Lane **4** is a pBR 322 plasmid DNA sample (containing both supercoiled and